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TCR Signaling: Another Abl-Bodied Kinase Joins the Cascade

Dispatch

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Protein tyrosine kinases have long been recognized as the most proximal actors in T-cell antigen receptor (TCR) signaling. Three non-receptor tyrosine kinase families (Src, ZAP-70 and Tec) are known to be critical, but a new study now shows that room needs to be made in this pathway for yet another protein tyrosine kinase family — Abl/Arg.

The ability of T cells to recognize and respond to infectious agents is a key factor in mounting an adaptive immune response, in which T and B cells work together with other components of the immune system to eliminate specific pathogens. Mature peripheral T cells rest in the G0 phase of the cell cycle. For an adaptive immune response to be initiated, T cells specific for the infectious agent need to be induced to proliferate and become activated. This signal is initiated by engagement of the T-cell antigen receptor by its ligand, a pathogen-derived antigenic peptide fragment presented to the T cell by an antigen-presenting cell.

Naturally, given the importance of the TCR in initiating T-cell activation, there has been intensive investigation of the signaling pathways that are initiated in response to TCR engagement. It was recognized as early as the mid-1980s that the earliest biochemical change that could be detected following engagement of the TCR was the rapid accumulation of phosphotyrosine on numerous cellular proteins. The primacy of protein tyrosine kinase activation in the TCR signaling cascade was subsequently firmly established by pharmacological, kinetic and genetic approaches, and tyrosine kinases remain the prime movers in most TCR signaling models [1].

None of the component polypeptide chains of the TCR complex have intrinsic tyrosine kinase activity. Instead, the TCR relies upon the recruitment of various non-receptor tyrosine kinases into the microenvironment of the activated TCR [1,2] (Figure 1). The Src-family kinases, Lck and Fyn were the first tyrosine kinases identified as playing a key role in TCR signaling. Lck (and possibly Fyn) are involved in the earliest TCR-initiated tyrosine phosphorylation events: the phosphorylation of tyrosine residues present within the immunoreceptor tyrosine-based activation motifs (ITAMs) present in each of the chains that make up the CD3 signaling module of the TCR. Once phosphorylated, the ITAMs act as docking sites, recruiting ZAP-70, via its tandem SH2 domains, to the activated TCR. This recruitment of ZAP-70 to the TCR places ZAP-70 into proximity with activated Lck, which then phosphorylates and activates ZAP-70. This then allows ZAP-70 to act on downstream signaling molecules without further regulation by other tyrosine kinases. Or so the model has gone for the past decade [3]. The results of Zipfel *et al.* [4] in this issue of *Current Biology*, however, indicate that things are somewhat more complicated, and that full ZAP-70 tyrosine phosphorylation and signaling events downstream of ZAP-70 require the activity of Abl/Arg-family tyrosine kinases [4] (Figure 2).

Leaving aside for the moment the obvious importance of providing a better understanding of the early events in TCR signaling, that this particular kinase, which has been studied for over two decades, and was one of the first proteins recognized to have tyrosine kinase activity (along with Src), should find a home at such a late date in such a well-studied signaling pathway seems truly remarkable [5]. However, the first clue that c-Abl might play a role in TCR signaling was actually uncovered in 1991, when it was found that targeted disruption of the *Abl1* gene in mice resulted in animals that had splenic and thymic atrophy and cell-autonomous lymphopenia [6,7]. Perturbed T-cell maturation, and the resultant lymphopenia, have been a hallmark of disrupted expression of most of the proteins identified as being critical components of the TCR signaling pathway, including Lck, ZAP-70, and the adaptor proteins LAT and SLP-76 [8].

What enabled these researchers to successfully examine the role of Abl/Arg in TCR signaling was a

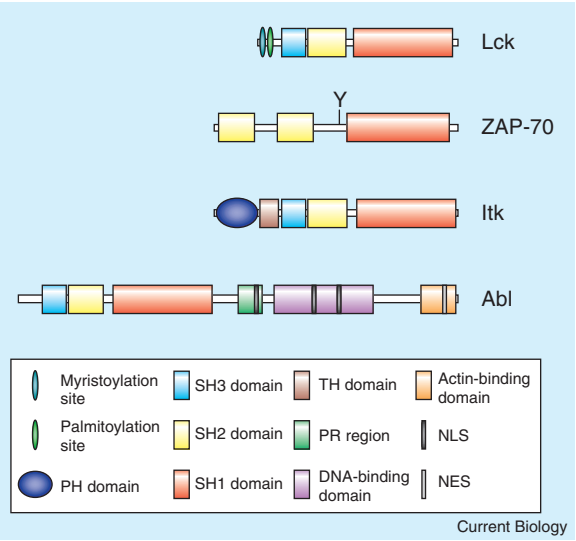

Figure 1. Domain structure of tyrosine kinases involved in TCR signaling.
The domain structures of the four tyrosine kinases that have been implicated in TCR signaling are shown, as is the tyrosine residue (Y) of ZAP-70 whose phosphorylation is regulated by Abl/Arg. PH, pleckstrin homology; SH, Src homology; SH1, the kinase domain; TH, Tec homology; PR, proline rich; NLS, nuclear localization sequence; NES, nuclear exclusion sequence.

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potent combination of a selective endogenous marker of Abl/Arg activity (phosphorylation of the downstream target CrkL on Tyr207), an Abl/Arg-selective pharmacological inhibitor (STI-571, also known as Imatinib or Gleevec™) and mice expressing various dosages of *Abl1* and *Abl2* (the genes encoding Abl and Arg, respectively). Having first established that TCR-stimulated phosphorylation of CrkL at tyrosine 207 was Abl/Arg-dependent (blocked by STI-571) and that Lck and Syk (a more kinetically active ZAP-70 family member) could not phosphorylate this residue *in vitro*, Zipfel *et al.* [4] used an antibody specific for the phosphorylated Tyr207 residue of CrkL to track Abl/Arg activity *in situ*, and found that TCR stimulation activated Abl/Arg. This finding was confirmed in *in vitro* immune complex kinase assays of plasma-membrane-bound (but notably not cytosolic or nuclear) c-Abl [4].

Having established that c-Abl is activated following TCR stimulation, delineation of where Abl/Arg fits into the TCR signaling cascade was established by both pharmacological and genetic approaches. STI-571 was used to determine which signaling steps downstream of TCR engagement were disrupted upon Abl/Arg inhibition. Importantly, STI-571 does not inhibit members of the Src and ZAP-70 family of tyrosine kinases [4,9]. Abl/Arg was found to act downstream of Lck, as STI-571 had no effect on CD3ζ chain and overall ZAP-70 tyrosine phosphorylation, events which require Lck activity. In addition, loss of Lck expression prevented TCR-stimulated c-Abl activation, while loss of ZAP-70 expression had no effect. While overall ZAP-70 tyrosine phosphorylation, as measured by anti-phosphotyrosine western blotting, was unaffected by STI-571, phosphorylation of Tyr319 of ZAP-70 was markedly reduced, as was tyrosine phosphorylation of LAT and SLP-76, two key ZAP-70 substrates [1,2]. Events downstream of LAT phosphorylation, including the recruitment of phospholipase C (PLC) γ1 to LAT, and the subsequent tyrosine phosphorylation of PLCγ1, and activation of the MAP kinase Erk were also defective in STI-571-treated T cells. In an elegant confirmation of the inhibitor results, mouse T cells genetically engineered to express Abl/Arg from only one of their four alleles showed substantial inhibition in TCR signaling to ZAP-70, LAT, PLCγ1 and Erk.

Thus, Abl/Arg would seem to act downstream of Lck and upstream of ZAP-70, although it is not yet clear whether Abl/Arg acts exclusively through ZAP-70, or whether it might directly target other TCR signaling proteins downstream of ZAP-70. For LAT phosphorylation in particular, ZAP-70 seems to be a required intermediary of c-Abl, as TCR stimulation did not cause LAT phosphorylation in the absence of ZAP-70 [10–12]. It is also not yet clear whether c-Abl acts by augmenting ZAP-70 activity per se (Tyr319 has long been considered to be a site of autophosphorylation [10,13,14]) or whether c-Abl directly phosphorylates this residue and thereby affects the ability of ZAP-70 to interact with other proteins. Relevant to this latter possibility, Tyr319 has been found to serve as a binding site for Lck and PLCγ1 [10,15], and lies within a consensus binding site for the SH2 domain of Crk family proteins [16] and of Abl itself [5].

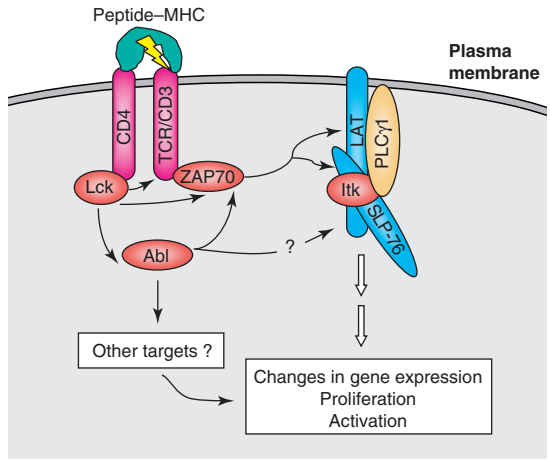

Figure 2. Placement of Abl/Arg in the TCR signaling pathway. Depicted are the early signaling events that lead to the formation of the multi-molecular 'signalosome' complex that forms in response to tyrosine phosphorylation of LAT and SLP-76. For graphical clarity the participation of the small molecular weight adaptor proteins Grb2 and Gads in the formation of the complex is not shown.

As would be expected in response to inhibition of the ZAP-70–LAT–SLP-76 signaling axis, STI-571 inhibition of Abl/Arg activity (or reduction in Abl/Arg gene dosage) also inhibited more distal TCR signaling events, including activation of the interleukin-2 (IL-2) gene promoter, IL-2 secretion and cell proliferation [4]. These results would predict that STI-571 has the potential to act as an immunosuppressant. This prediction has been borne out by recent reports of reactivation of latent Epstein–Barr and Varicella–Zoster viral infections in a subset of chronic myelogenous leukemia patients receiving treatment with STI-571 [17,18], providing eloquent testament to the significance of adding Abl/Arg to the cadre of TCR-activated tyrosine kinases, and of the importance of further delineating the mechanism by which Abl/Arg participates in TCR signaling.

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